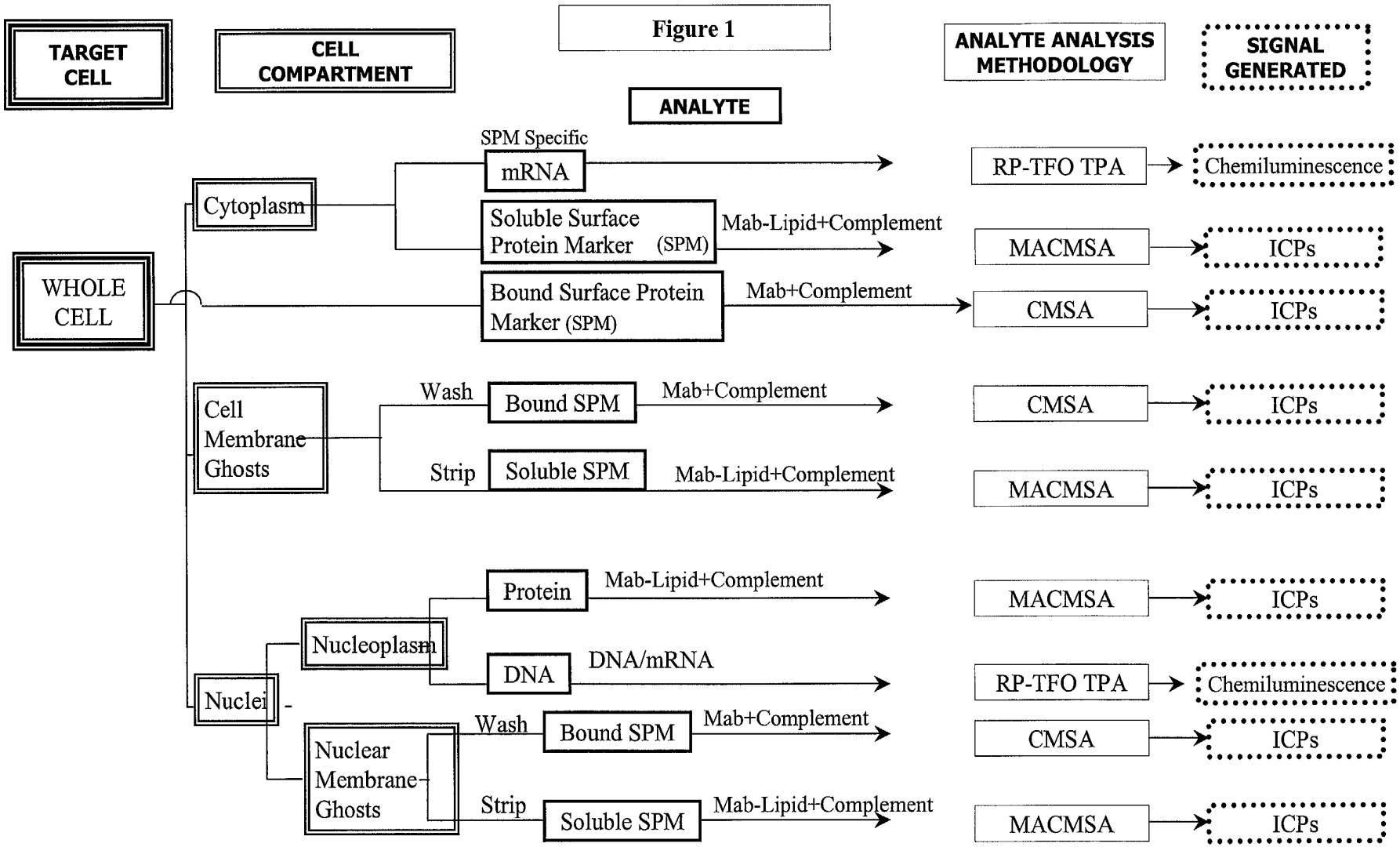
Figure 1


TARGET CELL
CELL COMPARTMENT
ANALYTE
ANALYTE ANALYSIS METHODOLOGY
SIGNAL GENERATED
WHOLE CELL
Cytoplasm
SPM Specific
mRNA
RP-TFO TPA
Chemiluminescence
Soluble Surface Protein Marker (SPM)
Mab-Lipid+Complement
MACMSA
ICPs
Bound Surface Protein Marker (SPM)
Mab+Complement
CMSA
ICPs
Cell Membrane Ghosts
Wash
Bound SPM
Mab+Complement
CMSA
ICPs
Strip
Soluble SPM
Mab-Lipid+Complement
MACMSA
ICPs
Nucleoplasm
Protein
Mab-Lipid+Complement
MACMSA
ICPs
Nuclei
DNA
DNA/mRNA
RP-TFO TPA
Chemiluminescence
Nuclear Membrane Ghosts
Wash
Bound SPM
Mab+Complement
CMSA
ICPs
Strip
Soluble SPM
Mab-Lipid+Complement
MACMSA
ICPs

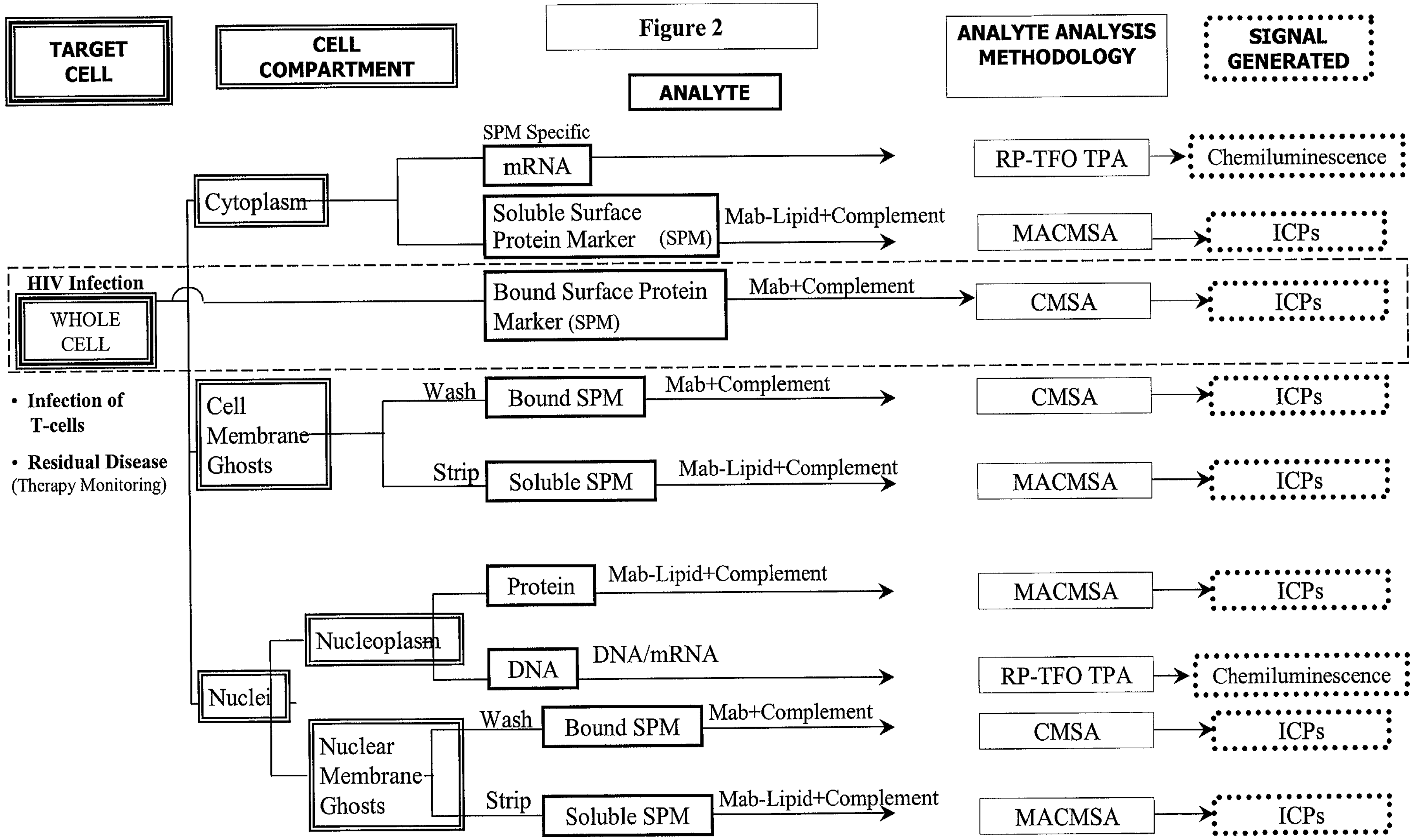

TARGET CELL
CELL COMPARTMENT
ANALYTE
ANALYTE ANALYSIS METHODOLOGY
SIGNAL GENERATED
SPM Specific
mRNA
RP-TFO TPA
Chemiluminescence
Cytoplasm
Soluble Surface Protein Marker (SPM)
Mab-Lipid+Complement
MACMSA
ICPs
HIV Infection
WHOLE CELL
Bound Surface Protein Marker (SPM)
Mab+Complement
CMSA
ICPs
• Infection of T-cells
• Residual Disease (Therapy Monitoring)
Cell Membrane Ghosts
Wash
Bound SPM
Mab+Complement
CMSA
ICPs
Strip
Soluble SPM
Mab-Lipid+Complement
MACMSA
ICPs
Protein
Mab-Lipid+Complement
MACMSA
ICPs
Nucleoplasm
DNA
DNA/mRNA
RP-TFO TPA
Chemiluminescence
Nuclei
Wash
Bound SPM
Mab+Complement
CMSA
ICPs
Nuclear Membrane Ghosts
Strip
Soluble SPM
Mab-Lipid+Complement
MACMSA
ICPs

Figure 2

Figure 3

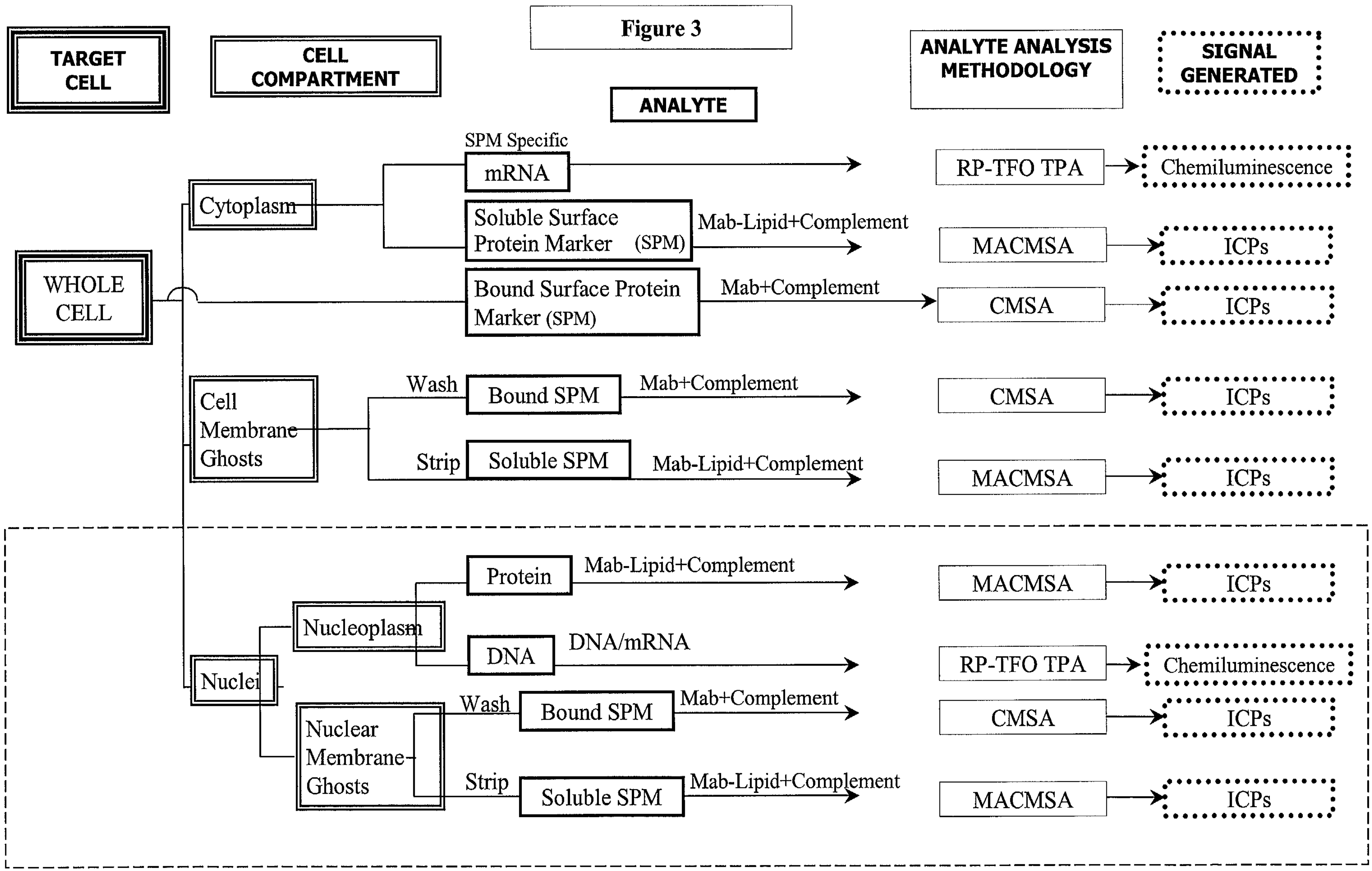
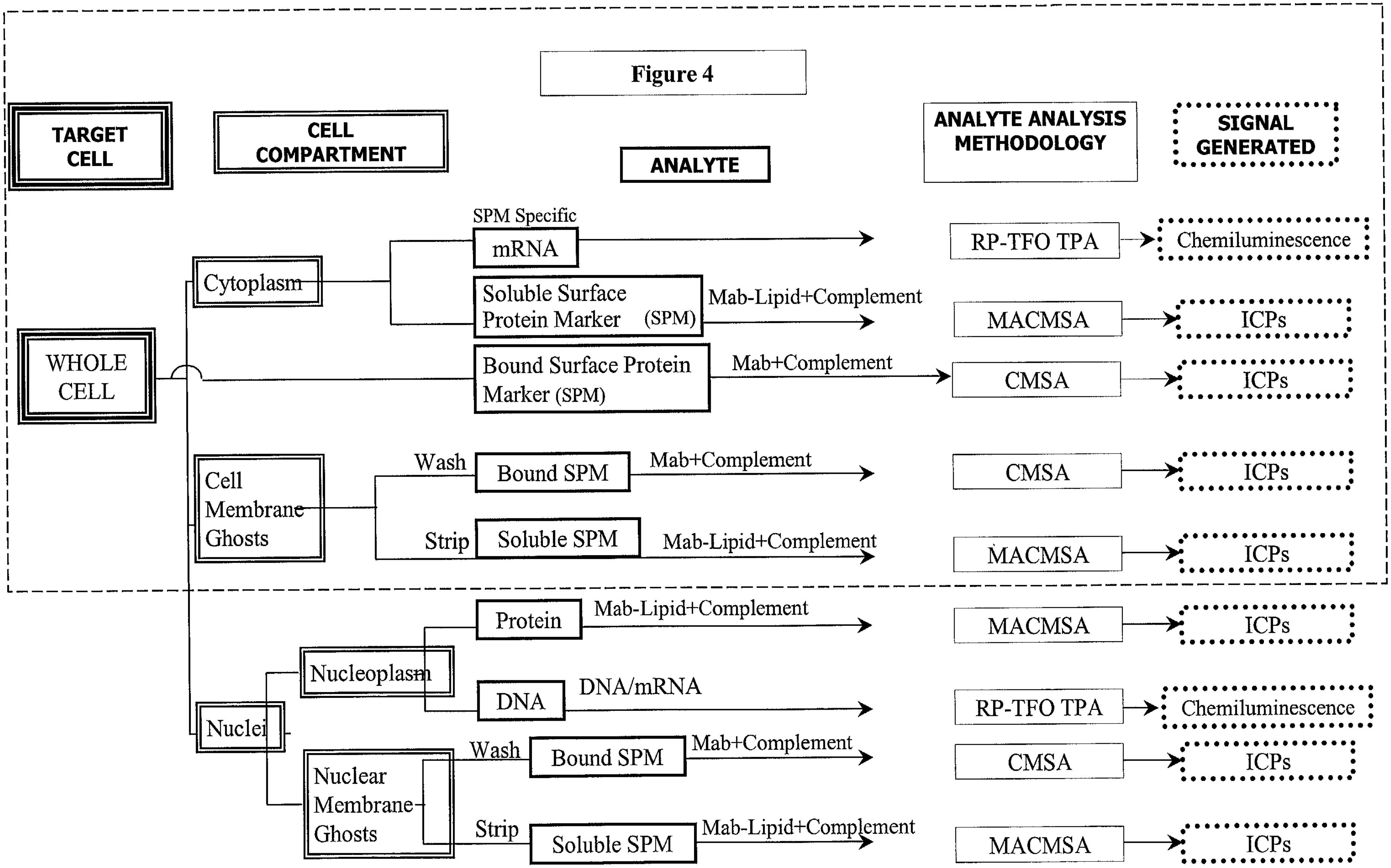

TARGET CELL
CELL COMPARTMENT
ANALYTE
ANALYTE ANALYSIS METHODOLOGY
SIGNAL GENERATED
WHOLE CELL
Cytoplasm
SPM Specific
mRNA
RP-TFO TPA
Chemiluminescence
Soluble Surface Protein Marker (SPM)
Mab-Lipid+Complement
MACMSA
ICPs
Bound Surface Protein Marker (SPM)
Mab+Complement
CMSA
ICPs
Cell Membrane Ghosts
Wash
Bound SPM
Mab+Complement
CMSA
ICPs
Strip
Soluble SPM
Mab-Lipid+Complement
MACMSA
ICPs
Nuclei
Nucleoplasm
Protein
Mab-Lipid+Complement
MACMSA
ICPs
DNA
DNA/mRNA
RP-TFO TPA
Chemiluminescence
Nuclear Membrane Ghosts
Wash
Bound SPM
Mab+Complement
CMSA
ICPs
Strip
Soluble SPM
Mab-Lipid+Complement
MACMSA
ICPs

Figure 4


TARGET CELL
CELL COMPARTMENT
ANALYTE
ANALYTE ANALYSIS METHODOLOGY
SIGNAL GENERATED
WHOLE CELL
Cytoplasm
SPM Specific
mRNA
RP-TFO TPA
Chemiluminescence
Soluble Surface Protein Marker (SPM)
Mab-Lipid+Complement
MACMSA
ICPs
Bound Surface Protein Marker (SPM)
Mab+Complement
CMSA
ICPs
Cell Membrane Ghosts
Wash
Bound SPM
Mab+Complement
CMSA
ICPs
Strip
Soluble SPM
Mab-Lipid+Complement
MACMSA
ICPs
Nuclei
Nucleoplasm
Protein
Mab-Lipid+Complement
MACMSA
ICPs
DNA
DNA/mRNA
RP-TFO TPA
Chemiluminescence
Nuclear Membrane Ghosts
Wash
Bound SPM
Mab+Complement
CMSA
ICPs
Strip
Soluble SPM
Mab-Lipid+Complement
MACMSA
ICPs

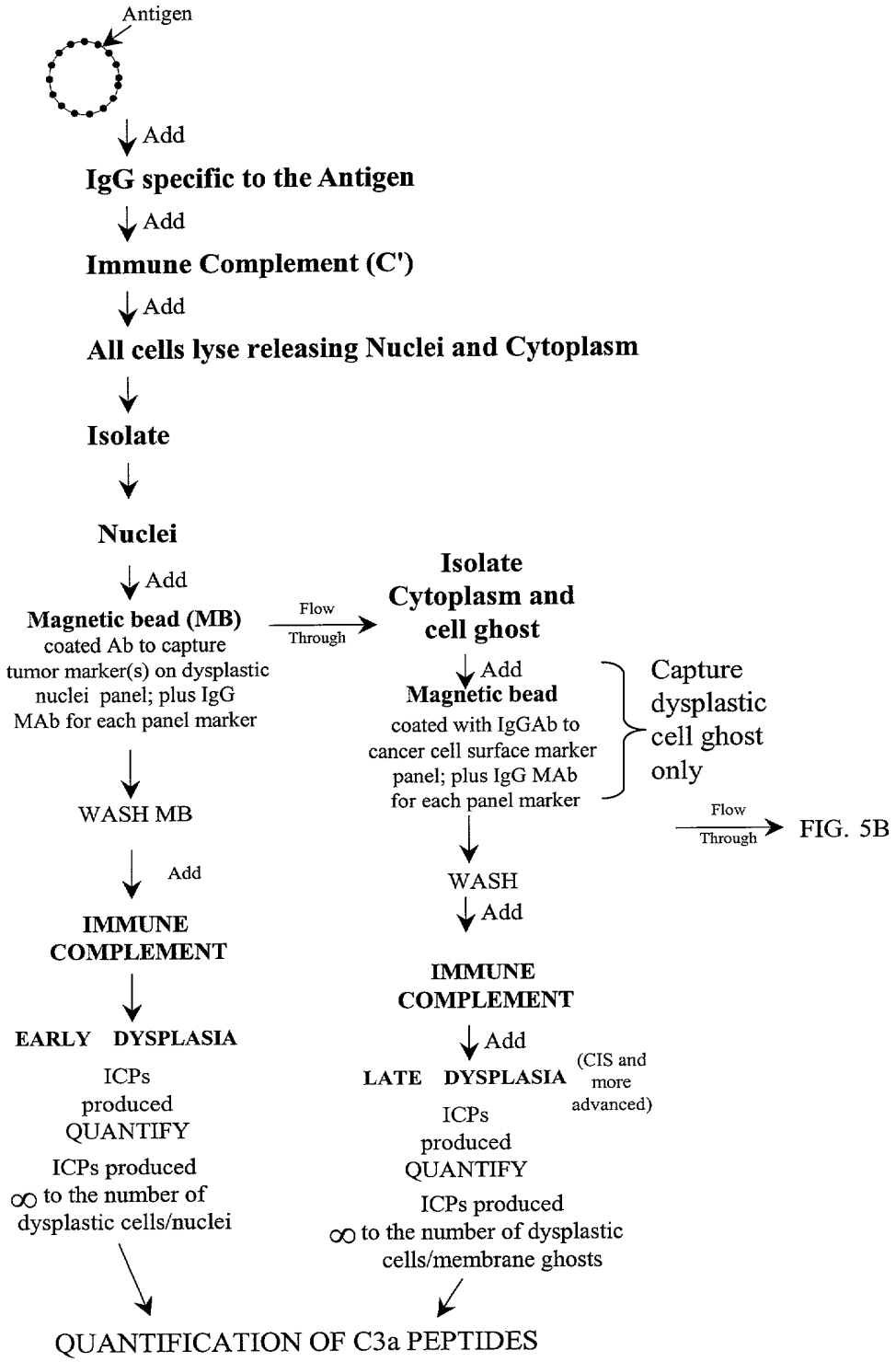

Antigen
Add
IgG specific to the Antigen
Add
Immune Complement (C')
Add
All cells lyse releasing Nuclei and Cytoplasm
Isolate
Nuclei
Add
Magnetic bead (MB)
coated Ab to capture tumor marker(s) on dysplastic nuclei panel; plus IgG MAb for each panel marker
Flow Through
Isolate
Cytoplasm and cell ghost
Add
Magnetic bead
coated with IgGAb to cancer cell surface marker panel; plus IgG MAb for each panel marker
Capture dysplastic cell ghost only
Flow Through
FIG. 5B
WASH MB
Add
IMMUNE COMPLEMENT
EARLY DYSPLASIA
ICPs produced QUANTIFY
ICPs produced ∞ to the number of dysplastic cells/nuclei
WASH
Add
IMMUNE COMPLEMENT
Add
LATE DYSPLASIA
(CIS and more advanced)
ICPs produced QUANTIFY
ICPs produced ∞ to the number of dysplastic cells/membrane ghosts
QUANTIFICATION OF C3a PEPTIDES

Figure 5A

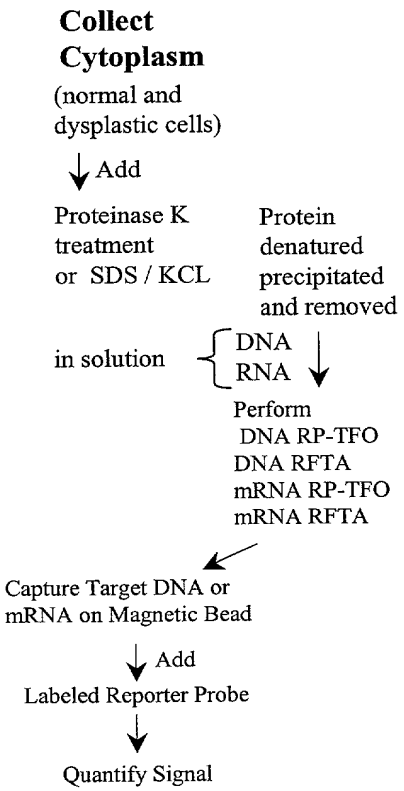

Collect
Cytoplasm
(normal and
dysplastic cells)
Add
Proteinase K
treatment
or SDS / KCL
Protein
denatured
precipitated
and removed
in solution
DNA
RNA
Perform
DNA RP-TFO
DNA RFTA
mRNA RP-TFO
mRNA RFTA
Capture Target DNA or
mRNA on Magnetic Bead
Add
Labeled Reporter Probe
Quantify Signal

Figure 5B

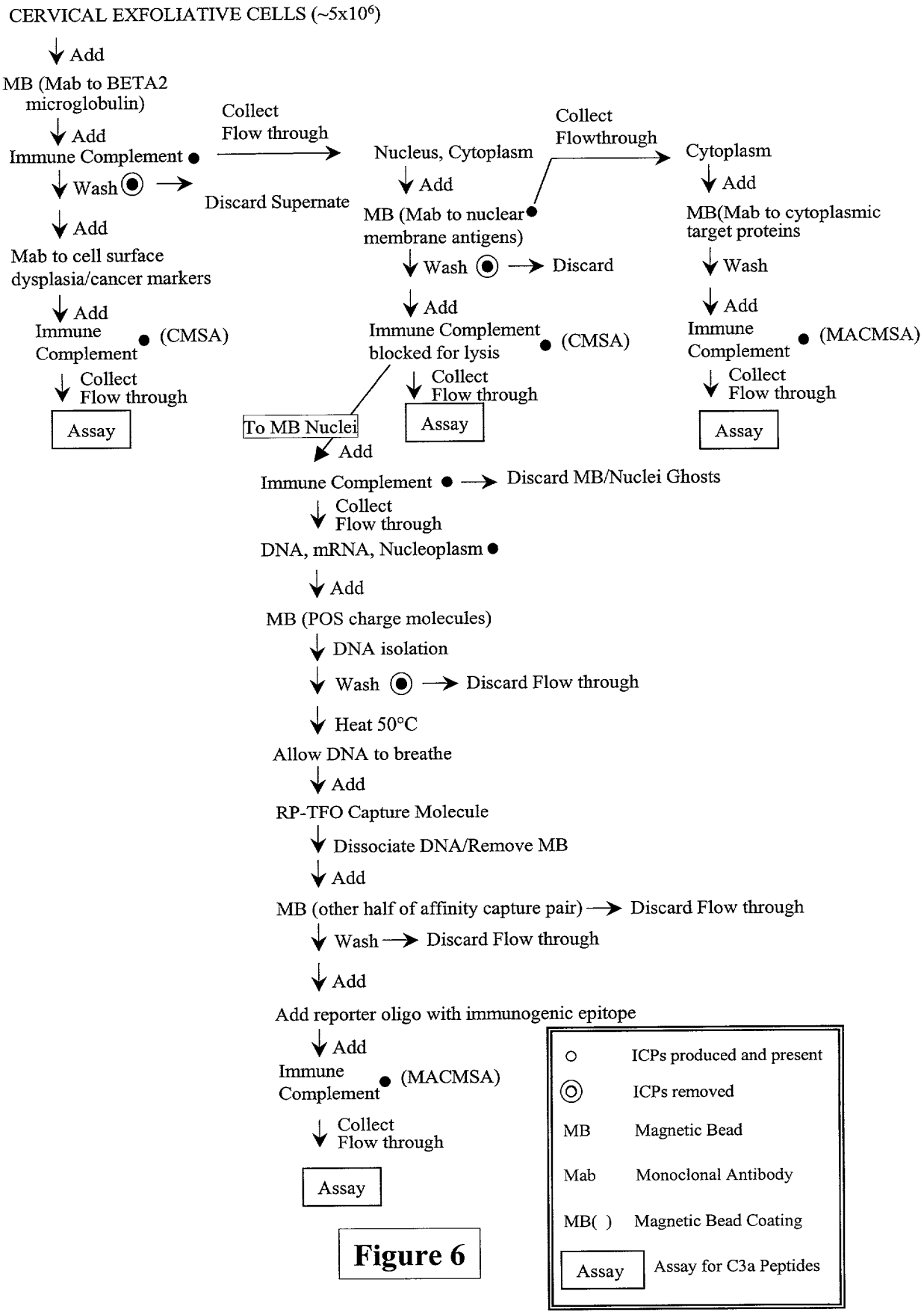

CERVICAL EXFOLIATIVE CELLS (~5x10⁶)
Add
MB (Mab to BETA2 microglobulin)
Add
Immune Complement ●
Wash ◉
Add
Mab to cell surface dysplasia/cancer markers
Add
Immune Complement ● (CMSA)
Collect Flow through
Assay
Collect Flow through
Discard Supernate
Nucleus, Cytoplasm
Add
MB (Mab to nuclear membrane antigens) ●
Wash ◉ → Discard
Add
Immune Complement blocked for lysis ● (CMSA)
Collect Flow through
Assay
Collect Flowthrough
Cytoplasm
Add
MB(Mab to cytoplasmic target proteins
Wash
Add
Immune Complement ● (MACMSA)
Collect Flow through
Assay
To MB Nuclei
Add
Immune Complement ● → Discard MB/Nuclei Ghosts
Collect Flow through
DNA, mRNA, Nucleoplasm ●
Add
MB (POS charge molecules)
DNA isolation
Wash ◉ → Discard Flow through
Heat 50°C
Allow DNA to breathe
Add
RP-TFO Capture Molecule
Dissociate DNA/Remove MB
Add
MB (other half of affinity capture pair) → Discard Flow through
Wash → Discard Flow through
Add
Add reporter oligo with immunogenic epitope
Add
Immune Complement ● (MACMSA)
Collect Flow through
Assay
○ ICPs produced and present
◉ ICPs removed
MB Magnetic Bead
Mab Monoclonal Antibody
MB( ) Magnetic Bead Coating
Assay Assay for C3a Peptides

Figure 6